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Primary cultures of human livers and their albumin-producing capacity.

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Abstract

Primary cultures of surgically obtained noncancerous portions of human liver tissues were made. Liver tissues were poorly dissociated with collagenase, but well dissociated with dispase. The yield and viability of cells were improved somewhat when dissociated with collagenase followed by dispase. The mean cell yield was 1.1 X 10(6) cells/g liver. The epithelial-like morphology of the dissociated liver cells was maintained for about one week, but thereafter degenerative alteration of cells was observed. In liver explant culture, an active outgrowth of cells was observed for more than one month. Albumin production in culture fluids from dissociated livers was detectable for about 2 weeks, but later became undetectable, while that from explant culture was detectable for at least one month. These data demonstrate that adult human hepatocytes can be isolated from noncancerous portions of livers with relatively high yield, and that albumin production of the dissociated cells is detectable for several days.

KEYWORDS: human liver cell culture, enzymatic dissociation, explant culture, albumin production

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Primary Cultures of Human Livers and Their Albumin-Producing Capacity

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Primary cultures of surgically obtained noncancerous portions of human liver tissues were made. Liver tissues were poorly dissociated with collagenase, but well dissociated with dispase. The yield and viability of cells were improved somewhat when dissociated with collagenase followed by dispase. The mean cell yield was 1.1×10^6 cells/g liver. The epithelial-like morphology of the dissociated liver cells was maintained for about one week, but thereafter degenerative alteration of cells was observed. In liver explant culture, an active outgrowth of cells was observed for more than one month. Albumin production in culture fluids from dissociated livers was detectable for about 2 weeks, but later became undetectable, while that from explant culture was detectable for at least one month. These data demonstrate that adult human hepatocytes can be isolated from noncancerous portions of livers with relatively high yield, and that albumin production of the dissociated cells is detectable for several days.

Key words: human liver cell culture, enzymatic dissociation, explant culture, albumin production

Numerous attempts to isolate adult human liver cells with or without perfusion have been reported (1-6). The use of normal liver tissues as experimental materials, however, is limited, especially for ethical reasons. Surgically removed hepatoma tissues often have noncancerous portions, which may contain not only hepatoma cells, but also normal and cirrhotic liver cells.

In the present communication, cell dissociation and primary cultures of the dissociated cells were studied using noncancerous portions of liver tissues. Their albumin-producing capacity was also compared to that of cells obtained by explant culture techniques that have routinely been used for the culture of adult human livers so far.

Materials and Methods

Human liver tissues were obtained from surgical specimens which consisted of hepatoma and nonhepatoma portions. The nonhepatoma portions were placed in ice-cold Hanks' solution, cut into smaller fragments and separated into two parts. The production of α-fetoprotein of nonhepatoma portions of livers used in the present study was examined by enzyme-linked immunosorbent assay (ELISA) after 2 days in primary culture as described below. No α-fetoprotein was detected, suggesting that few hepatoma cells were present in the tissues used. Some of the fragments were submitted to explant culture, and others were digested with 0.05% collagenase (type I, Sigma), 1000 U/ml dispase (Godo-Shusei, Chiba, Japan) or 0.05% collagenase followed by 1000 U/ml dispase. The cell suspensions thus obtained were filtered and centrifuged 3 times at 50x g for 30

min. The pellet was resuspended in a growth medium and subjected to primary culture. The growth medium consisted of RPMI 1640 supplemented with 5 μg/ml insulin, 5×10^{-7}M hydrocortisone, 0.2% lactalbumin hydrolysate and 5 or 20% bovine serum (BS).

For the detection of albumin production, cells (7×10^4 cells/well) were inoculated into 24 multiwell cluster dishes (Falcon) with or without a coating of 30 μg/ml type I collagen (Nitta-Gelatine Co., Osaka, Japan) for 1 h. The albumin secreted into the medium was detected by ELISA using rabbit antihuman and horseradish peroxidase conjugated rabbit antihuman antibodies (Cappel, Cochranville, PA)(7).

Results

The dissociation procedure was used on liver fragments from 3 patients. The tissues were poorly dissociated into single cell suspensions with 0.05% collagenase. With 1000 U/ml dispase for 40-70 min, however, all the tissues samples examined were well dissociated into single cell suspensions. When the tissues were treated with collagenase followed by dispase, cell yield and viability were somewhat higher than when treated with dispase alone (Table 1). The mean cell yield obtained by collagenase followed by dispase treatment was 1.1×10^6 cells/g wet weight of liver.

Enzymatically dissociated and nondissociated tissues were submitted to primary culture. The epithelial-like morphology of the dissociated cells was maintained for about one week, but thereafter degenerative alteration including an increase in floating cells with cytoplasmic granules, which were found not to be viable by dye-exclusion test, were observed. This phenomenon was more remarkable in culture with 20% BS than with 5% BS. On the other hand, in explant cultures, an outgrowth of epithelial-like cells was observed for more than one month.

Table 1 Enzymatic dissociation of human liver tissues[a]

Dissociation		Cell yield	
Enzyme	Time (min)	No. of cells ($\times10^5$/g liver)	Viability (%)
Exp 1			
Collagenase	15	1.1	6.7
	25	3.5	14.7
Dispase	40	3.2	33.3
	70	2.8	37.5
Collagenase[b]	25		
Dispase	40	6.3	49.3
	70	6.5	49.3
Exp 2			
Collagenase	15	very few	—
	25	very few	—
Dispase	40	9.0	23.5
	70	9.0	22.9
Collagenase[b]	25		
Dispase	40	17.6	—
	70	16.8	25.7
Exp 3			
Collagenase	15	9.8	3.0
	25	4.9	1.0
Dispase	40	8.9	32.4
	70	12.8	53.2
Collagenase[b]	25		
Dispase	40	8.2	40.4
	70	12.9	53.2

a: Collagenase (0.05%) or dispase (1000 U/ml) was used.

b: Digested by collagenase for 25 min followed by dispase for 40 to 70 min.

Mitotic activity was infrequently observed (Fig. 1).

Albumin production in culture fluids from dissociated liver cells was detectable for about 2 weeks, but later became undetectable regardless of the presence or absence of hydrocortisone. The rate of decrease of albumin production was lower in collagen-coated culture dishes than in uncoated ones (Fig. 2). On the other hand, in the explant culture, the levels of albumin secreted in spent medium were 37.5, 165 and 115 ng/ml/72 h after 15, 19 and 33 days in primary culture, respectively.

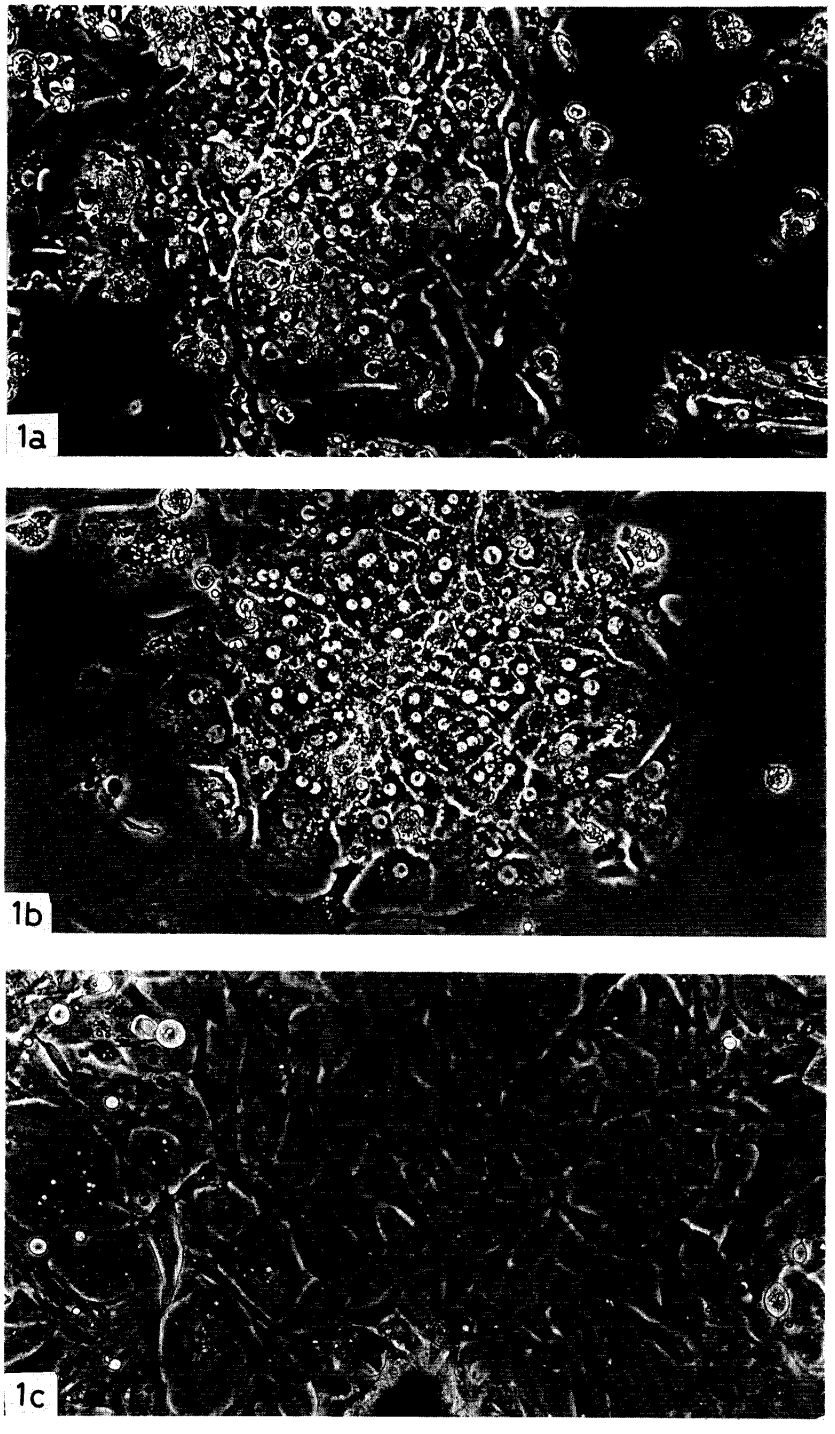

Fig. 1 Morphology of human liver cells in primary culture. 1a & 1b, Enzymatically dissociated liver cells cultured for 6 days in RPMI-1640 supplemented with 20% BS (1a) and 5% BS (1b). 1c, Outgrowth of cells from a liver explant maintained for 56 days in RPMI-1640 supplemented with 20% BS. Phase contrast. ×100.

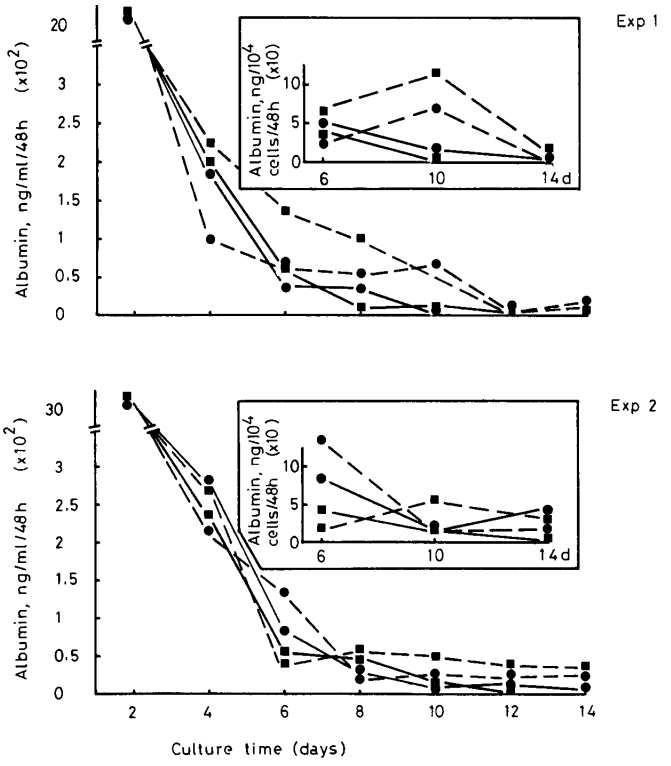

Fig. 2 Time course of albumin secretion in cultured human liver cells. Dissociated human liver cells were inoculated onto collagen-coated dishes with (■--■) or without (●--●) hydrocortisone, and uncoated dishes with (■—■) or without (●—●) hydrocortisone. The insert shows the albumin contents/10^4 cells/48h.

Discussion

Many studies have been carried out concerning mechanical or enzymatic cell dissociation of normal liver tissues obtained surgically from adult humans not exhibiting any liver lesion to obtain a higher hepatocyte yield (1, 2, 5, 6). In these studies, cell yields of more than 2×10^6 cells/g wet weight of liver were obtained. Gugen-Guillouzo *et al.* reported the isolation of as many as $1-10\times10^8$ human adult hepatocytes by perfusing the left lobe of the liver (2). In the present study, a relatively high cell yield (1.1×10^6 cells/g liver) was obtained by collagenase followed by dispase-treatment. The results suggest that non-cancerous portions of human livers, which may contain a number of degenerative hepatocytes, are usable for studies of primary culture.

Many attempts to culture normal adult hepatocytes have been made, but hepatocyte specific products have been detected only for several days (3). In the present study, albumin production of enzymatically dissociated liver cells was detectable only for several days, while that of cells in explant cultures was detectable for at least one month. These findings suggest that not only the dissociating enzymes used but also culture conditions such as culture medium, serum or substratum are not suited to the long term culture of the dissociated hepatocytes. On the other hand, in explant cultures, it is likely that intact hepatocytes are continuously supplied by peripheral outgrowth of cells around the small liver fragment, and therefore differentiated functions such as albumin production are maintained well for longer periods of time.

References

1. Ballet F, Bouma M-E, Wang S-R, Amit N, Marais J and Infante R: Isolation, culture and characterization of adult human hepatocytes from surgical liver biopsies. Hepatology (1984) **4**, 849-854.
2. Guguen-Guillouzo C, Campion JP, Brissot P, Glaise D, Launois B, Bourel M and Guillouzo A: High yield preparation of isolated human adult hepatocytes by enzymatic perfusion of the liver. Cell Biol Int Rep (1982) **6**, 625-628.
3. Guilly YL, Lenoir P and Bourel M: Production of plasma proteins by subcultures of adult human liver. Biomedicine (1973) **19**, 361-364.
4. Lazizi Y, Guillon JC and Pillot J: Long term cultivation of functionally active normal human adult hepatocytes. Dev Biol Stand (1983) **54**, 75-79.
5. Maekubo H, Ozaki S, Mitmaker B and Kalant N: Preparation of human hepatocytes for primary culture. In Vitro (1982) **18**, 483-491.
6. Miyazaki K, Takaki R, Nakayama F, Yamauchi S, Koga A and Todo S: Isolation and primary culture of adult human hepatocytes. Cell Tissue Res (1981) **218**, 13-21.
7. Uotila M, Rouslahti E and Engvall E: Two site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. J Immunol Methods (1981) **42**, 11-13.

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